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CYP1B1 Polymorphism as a Risk Factor for Race-Related Prostate Cancer

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14. ABSTRACT

In the first hypothesis, CYP1B1 expression in Caucasian prostate cell lines were increased in cancerous (DU145 & PC-3) compared to normal (RWPE-1) cells. Analysis of expression in human tissue cDNAs from Caucasians also showed higher levels of CYP1B1 in cancer compared to BPH. CYP1B1 protein was present in both races and though not significant, generally was higher in tumor regions compared to normal adjacent regions for both African-Americans and Whites. In the second hypothesis, racial differences for CYP1B1 polymorphisms are observed as allele frequencies for the variant at codons 119 and 432 are greater among Blacks ($P<0.001$) whereas the 453 variant is predominant in Whites ($P<0.001$). Within race, a case control study show the variant at codon 453 plays a protective role for PC among Blacks ($P<0.05$). Interestingly, SNPs at codons 432 and 449 are determined to be linked and the 432G-449C haplotype was observed to be a risk for PC ($P<0.05$). In a sampling of cases, no differences were observed between stages (<T2c vs >T2c) and grades (<7 vs >7) of PC in either race. In the remaining year, more SNP studies with additional samples to be collected; as well as further experimentation with aim #1 will be performed.

15. SUBJECT TERMS
CYP1B1, Polymorphism, Prostate cancer, Race-related

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INTRODUCTION

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of death among men with cancer in the USA. When comparing races, the incidence and mortality rates of prostate cancer in African-Americans is higher than in Caucasians and Asians. Cytochrome P450 (CYP) 1B1 converts estrogens to the 4-hydroxy-catechol-estrogens. Studies show this catechol-estrogen to be mutagenic and may lead to prostate cancer. Polymorphisms of CYP1B1 have been associated with various types of cancers and we have shown that CYP1B1 polymorphisms have higher risks for prostate cancer (Abstract; J. Urol. 171(Suppl. 4):111, 2004). However, such studies are lacking in race-related prostate cancer. There are at least 4 polymorphisms that have been identified on the CYP1 B1 gene that results in a structural change in the enzyme and are at the following locations: codons 48 (C to G), 119 (G to T), 432 (C to G), and 453 (A to G). The main goal of this project is to investigate whether polymorphisms of the CYP1B1 gene can be a risk factor for race-related prostate cancer. To determine this, two specific aims are tested. In specific aim #1, the hypothesis that CYP1B1 gene is hyper-activated during malignant transformation of race-related prostate cells is tested. In the 2nd aim, the hypothesis that single nucleotide polymorphisms (SNPs) of the CYP1B1 gene have higher risk for race-related prostate cancer and correlate with hyper-activated CYP1B1 gene is tested. Data generated from these experiments will determine whether CYP1B1 gene expression differs between Caucasian and African-American prostate cancer samples. Also, these experiments will determine whether CYP1B1 SNPs are involved in race-related prostate cancer. This knowledge will help to understand the genetic basis for racial differences as well as identify the subjects who are at higher risk for prostate cancer.

BODY

Samples: Unless specified, all specimens reported are from our previously funded projects that are pre-existing, archival specimens from African-Americans and Caucasians. cDNA arrays and cell lines are from commercial sources. Experimental results are based on these samples.

Task #1. To determine if the CYP1B1 gene is differentially expressed between races (African-Americans and Whites) and in different stages and grades of prostate cancer.

CYP1B1 in prostate cell lines from Whites.

Cell lines were utilized to see if there are differences in CYP1B1 expression between cancerous versus normal cells. Prostate cell lines of Caucasian origin were utilized and obtained from ATCC (Manassas, VA). Cancerous cells (DU 145 and PC-3) were cultured in RPMI 1640 (UCSF Cell Culture Facility; San Francisco, CA) with 10% fetal bovine serum (Atlanta Biologicals; Atlanta, GA). Normal cells (RWPE-1) was cultured in Keratinocyte-SFM +L-Glutamine, +EGF and BPE (GIBCO/Invitrogen; Carlsbad, CA). From the prostate cell lines, total RNA was extracted using the Qiagen RNeasy kit according to manufacturer's instructions. Quantity and quality of RNA was measured at 260 and 280 nm with the use of a spectrophotometer. RNA was reverse-transcribed to cDNA using the Promega Reverse Transcription System (Madison, WI). To measure CYP1B1 expression in cell lines, cDNA were amplified by PCR and electrophoretically separated on 2% agarose gels using 200 volts at ambient temperature. The products were then visualized by ethidium bromide staining under UV light and image taken on an Alpha Imager Gel Documentation System (Alpha Innotech, San

Leandro, CA). Quantitative real-time PCR was performed using the ABI Prism 7500 Fast Thermal Cycler (Applied Biosystems Inc.; Foster City, CA). cDNA was amplified using TaqMan Fast Universal PCR Master Mix, RNAse-free water and primers specific to either the CYP1B1 gene or glyceraldehyde-3-phospate dehydrogenase (GAPDH), as internal control. Primers were obtained from Applied Biosystems (Foster City, CA. Catalog Numbers: Hs00164383_m1, and Hs99999905_m1). Samples were replicated four times. Results were tabulated using 7500 Fast System SDS Software version 1.3.1.

Figure 1 shows the gel electrophoresis products of CYP1B1 and GAPDH. CYP1B1 is observed to be expressed in both normal (RWPE-1) and cancerous (DU145 and PC-3) prostate cell lines from Caucasians. Quantitation of CYP1B1 by the real-time PCR in these cell lines is shown in Figure 2. A dramatic 14.5-fold increase of CYP1B1 expression in the PC-3 cancer cell line, and a 17.7-fold increase in the DU145 cancer cell line were observed, as compared to the normal cell line RWPE-1.

CYP1B1 RNA in prostate tissues.

Since we find an increase of CYP1B1 in prostate cell lines, we next evaluated CYP1B1 expression in human prostate tissue. We performed PCR amplification and real-time PCR analyses on cDNA acquired from 95 different prostate tissues using TissueScan Prostate Tissue qPCR Arrays (OriGene Technologies; Rockville, MD). These arrays consisted of 11 samples from individuals with hyperplasia of the prostate, 20 samples from prostate lesions, and 49 samples from prostate tumors. Lesional cells are those that are neither cancerous, nor are they described as "within normal limits". Information about race/ethnicity was provided for 58 of the patients, of which 3 were Asian, 5 were African American, and 50 were Caucasian. Total Gleason scores ranged from 4 to 9, and cover disease stages I through IV.

Results of PCR amplification run on gels is shown in Figure 3. CYP1B1 expression was confirmed in White BPH, White cancer and Black cancer. Since expression were observed, quantitative real-time RT-PCR analysis was performed on these samples. Figure 4 shows the relative levels of CYP1B1 expression in BPH, lesion, and tumor. Transcripts were detectible in all samples and we observed elevated levels of CYP1B1 in lesion (1.80-fold) and tumor tissues (2.40-fold), as compared to BPH. While insufficient sample size prevented a thorough analysis by race, the same pattern of expression was also observed among White patients, with lesion having a 1.51-fold increase and tumor having a 3.29- fold increase compared to BPH (Figure 5). Interestingly, we observed a slightly higher (1.36-fold) expression of CYP1B1 in tumor tissues from White patients as compared to African Americans (Figure 6). We found no clear correlation between the expression levels and Gleason scores.

CYP1B1 protein in prostate tissues.

As demonstrated above, we find CYP1B1 to be expressed in both cell lines and human prostate tissues, with RNA levels higher in prostate tumors compared to BPH tissue or normal cell lines. We then investigated CYP1B1 protein in prostate tissue. Prostate cancer tissue samples were obtained from 15 Caucasian and 15 African American males living in the San Francisco Bay Area whom had undergone radical prostatectomy with a pathologically confirmed diagnosis of prostate carcinoma at the Veterans Affairs Medical Center (VAMC), San Francisco, Department of Urology. Specimens were also obtained from 15 Caucasian and 15 African American men living in the bay area who had pathologically confirmed benign prostatic hypertrophy (BPH). Relevant clinico-pathologic data (age, Gleason grade, and tumor-node-

metastasis stage) were collected from the patient files. The average age for Caucasians with prostate cancer, African Americans with prostate cancer, Caucasians with BPH and African Americans with BPH were 69.9, 67.5, 72.8 and 66.1 years, respectively. All tissues were formalin-fixed and embedded in paraffin. All specimens were archived, de-identified, pre-existing specimens.

Paraffin embedded tissue samples were cut into 5 micron sections and placed onto silanized slides (Dako, Carpinteria, CA). The slides were then incubated at 60ºC overnight. To deparaffinize, slides were treated with xylene three times for 5 minutes each, then 100% ethanol for 10 minutes, 95% ethanol for 10 minutes, 70% ethanol for 5 minutes, and washed in deionized water for one minute. To unmask antigens, slides were then placed in citrate buffer (LabVision; Fremont, CA) and heated in a 900W microwave on high for 7 minutes. After allowing to cool, endogenous peroxidase activity was quenched by soaking slides in 3% hydrogen peroxide for 8 minutes (Sigma-Aldrich; St. Louis, MO). Blocking serum from ImmunoCruz Staining System (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was used minimize non-specific binding. CYP1B1 polyclonal rabbit antibody (Abcam Inc., Cambridge, MA) was diluted 1:500 in serum block and slides were incubated overnight at 4ºC. Unbound primary antibody was removed by washing with phosphate-buffered saline (PBS) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) twice. Biotinylated secondary antibody from the staining kit was added and incubated for 30 minutes. Slides were then washed twice in PBS and incubated for 30 minutes with horse radish-Streptavidin reagent. Diaminobenzidine Plus Substrate (UltraVision Plus System; Labvision; Fremont, CA) was added as a chromogen until staining became visible. Finally, slides were washed and counterstained with hematoxylin. After staining, each section of tissue was visually inspected under light microscopy at 100x magnification, and each field was ranked on an overall scale from 0 to 3; with 0 indicating the absence of immunostaining; 1, weak staining; 2, moderate staining; and 3, strong staining. A composite score was then calculated based on a weighting of the number of fields that were strong, moderate, weak or none. Staining scores are given in terms of (mean $\pm$ standard deviation).

Tissues from prostate cancer patients were inspected by a pathologist and marked for tumor regions and regions that were normal adjacent to tumor (NAT), which was scored separately. Figure 7 shows CYP1B1 protein staining in cancer region, BPH, and NAT for African-Americans and Caucasians. CYP1B1 protein was observed to be localized in the cytoplasm of prostate cancer cells with some expression in smooth muscle cells. While staining intensity varied considerably between individuals, overall the most intense staining was found in tumor areas (1.53 $\pm$ 0.71), followed by BPH (1.45 $\pm$ 0.5), and normal adjacent (1.32 $\pm$ 0.74). When comparing between races, there were high variation leading to large standard deviation but overall, highest expression of CYP1B1 was found in the tumor regions of African American samples (1.57$\pm$ 0.87) and weakest in the normal regions of Caucasian samples (1.2 $\pm$ 0.73), Table 1.

Task #2. To determine if single nucleotide polymorphisms (SNPs) of the CYP1B1 gene are risk factors for the etiology of race-related prostate cancer and correlate with hyper-activity of its gene.

From the pre-existing BPH and prostate cancer obtained from African-American and Caucasian patients, and normal healthy from African-American, DNA was collected by using a DNA extraction kit (Qiagen, Valencia, CA). Quantity and quality of DNA was measured at 260 nm and 280 nm by the use of a spectrophotometer. A two-step polymerase chain reaction (PCR) procedure was designed for the analysis of CYP1B1 polymorphisms. The primers of four of the polymorphic sites studied so far (codons 119, 432, 449, and 453) and PCR conditions are summarized in Table 1. In the first PCR, DNA (10 ng) was amplified in a 20 ul reaction containing 1.5 mM $MgCl_2$, 0.8 mM dNTP mix, PCR buffer, and 0.5 units of Red-Taq polymerase (Sigma-Aldrich, St. Louis, MO), along with primer sets designed to contain the polymorphic sites (Table 2). In the sequence-specific PCR (SSP), each polymorphic fragment was further amplified under similar conditions as the first-step PCR except for the use of SSP primer sets (Table 2). Each of the SSP products were electrophoretically separated on 3% agarose gels using 200 volts at ambient temperature. The products were then visualized by ethidium bromide staining under UV light and recorded in the Alpha Imager Gel Documentation System. To confirm genotyping, products of the first PCR were subjected to direct DNA sequencing. In the case of the codon 449 polymorphism, direct sequencing was performed on all samples. DNA was purified from gels using a QIAquick PCR purification kit (Qiagen; Valencia, CA). Sequence analysis of purified products was then determined by using the first PCR primers and ABI 377 Sequencer and Dye Terminator Cycle sequencing kit (Applied Biosystems Inc.; Foster City, CA). Confirmation of DNA sequence was done on at least 3 representative samples for each of the polymorphic types. Frequencies of the various genotypes and allele types of CYP1B1 polymorphisms in the different categories of samples were determined and tabulated. Chi-square analysis was used to test each of the polymorphisms for differences in genotypic and allelic frequencies between Whites and Blacks as well as between BPH and prostate cancer. Relative risk associated with a particular genotype or allele was estimated by calculating odds ratios (OR) along with 95% confidence intervals (CI). In African-American samples, further analyses included case-control, linkage disequilibrium, and haplotype evaluation.

Results of the genotypic and allelic frequencies of the three SNP sites of the CYP1B1 gene between African-Americans and Caucasians for BPH and prostate cancer, and total patients (including African-American controls) are shown in Tables 3 and 4, respectively. Interestingly in total patients, the variant genotype at all 3 codons differ significantly between African-Americans and Caucasians. The T/T genotype at codon 119 are highly predominant in African-Americans as compared to Caucasians (Chi-square, $P<0.001$). OR (95% CI) were 2.71 (1.61-4.55) and 3.55 (1.96-6.43) for the G/T and T/T genotypes, respectively, in Blacks compared to Whites. At codon 432, the C/G and G/G genotypes were much greater in African-Americans with OR (95% CI) values of 3.90 (2.03-7.52) and 12.09 (5.95-24.58) as compared to Caucasians (Chi-square, $P<0.001$). The polymorphism as codon 453 also proved to be different between Blacks and Whites although the variant A/G and G/G genotypes were much greater in Caucasians (Chi-square, $P<0.001$). Thus the OR (95% CI) values were lower for Blacks with values of 0.17 (0.09-0.34) for A/G and 0.10 (0.01-1.02) for the G/G genotypes as compared to Caucasians. Significant differences in genotype frequencies between Blacks and Whites also occurred when data were evaluated just among the prostate cancer patients at all 3 codons (Table 3). When classified among the BPH patients, significant differences were observed at the codon

432 and 449 sites but not the 119 site (Table 3). This may be due to the small N size of BPH patients.

Likewise when evaluating allele frequencies between African-Americans and Caucasians, significant differences were observed at all 3 SNP sites in total patients ($P<0.001$; Table 4). This was also true in prostate cancer patients at all 3 sites ($P<0.001$; Table 4). In BPH patients, significance was only found at the codon 432 site and lack of significance for other two sites may be due to the small BPH patient N size.

A case-control study was performed on African-American as 97 healthy controls and 97 age-matched prostate cancer specimens were obtained. In addition to the 3 polymorphic sites, the codon 449 SNP site was also evaluated. Table 5 shows the results of genotypic and allele frequencies in prostate cancer and controls at these 4 polymorphic sites. The polymorphism at codon 453 was relatively low in frequency as the homozygous variant (G/G) was not observed in any patient. Frequencies in healthy controls were in Hardy-Weinberg equilibrium at these sites. When compared to healthy volunteers, no differences in polymorphic frequencies were observed in prostate cancer cases at codons 119, 432, and 449. However, the SNP at codon 453 proved to play a protective role as cancer cases had a significantly lower frequency of the variant A/G genotype when compared to healthy controls ($P=0.035$). The OR was 0.16 with a 95% CI of 0.05 to 0.51 for the A/G genotype as compared to A/A. In concordance, the allele frequency also differed with the variant G being much lower in prostate cancer cases ($P=0.024$).

Linkage disequilibrium analysis was performed to determine any linkage between the 4 polymorphic sites. Healthy control samples were analyzed and interestingly, a strong linkage was observed between the codon 432 and codon 449 sites (Table 6). The D-value between these two sites were 0.1905. No linkages were observed between other polymorphic sites. Since these two sites were linked, haplotype analysis was performed between prostate cancer cases and controls. Interestingly as shown in Table 7, the 432G – 449C haplotype proved to be significantly associated for cancer as 6.3% of this combination was found in cases compared to just 1.0% in controls ($P=0.016$).

Clinical and pathological information were obtained for a portion of the prostate cancer patients. Cancer samples were classified in terms of stage and due to the small N size when divided between races, stage classifications were made as $< T2c$ or $\geq T2c$. Results for each race is shown in Table 8. No differences were observed between stages for either race although sample size is small. Likewise, cancer samples were classified in terms of pathological grade and were based as < 7 or ≥ 7. Table 9 shows the results of samples based on grade for both Blacks and Whites and no differences were observed.

Figure 1. Gel electrophoresis of GAPDH and CYP1B1 in Caucasian prostate cell lines (RW PE-1, DU145 and PC-3)

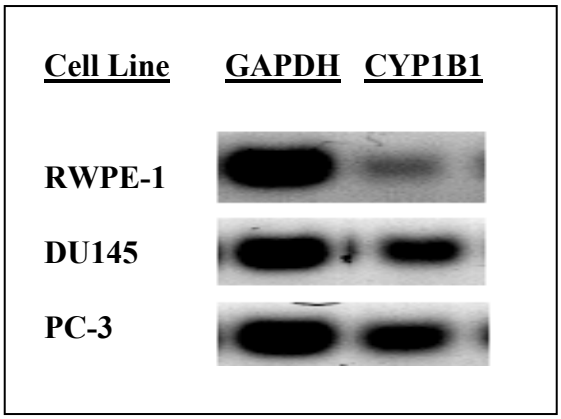

Figure 2. Relative expression levels of CYP1B1 in Caucasian prostate cell lines (RW PE-1, DU145 and PC-3). Values normalized to RWPE-1 control cells.

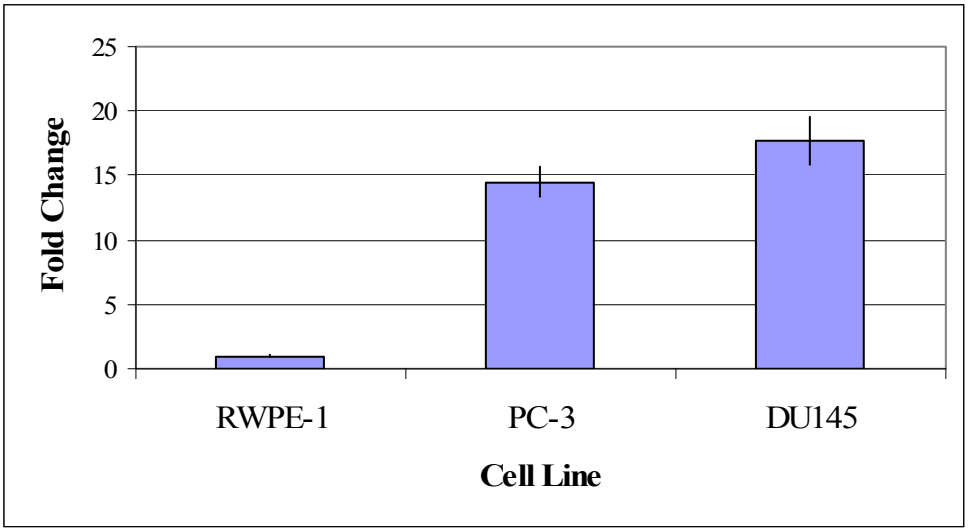

Figure 3. Gel electrophoresis of CYP1B1 and GAPDH in African-American prostate tumor, Caucasian prostate tumor, and Caucasian BPH.

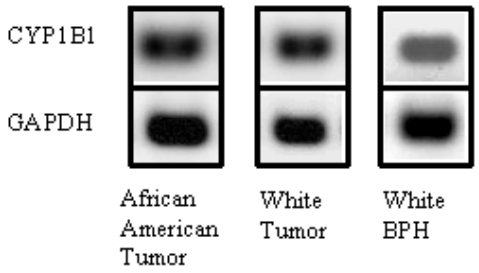

Figure 4. Relative expression levels of CYP1B1 in human BPH, prostatic lesion, and prostate tumor. African-Americans and Caucasians combined. Values normalized to BPH.

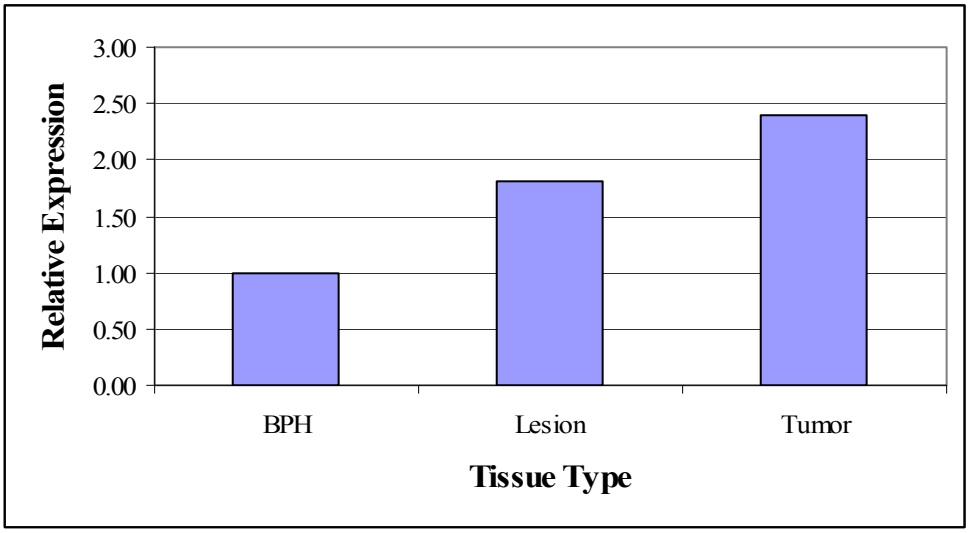

Figure 5. Relative expression levels of CYP1B1 in BPH, prostatic lesion, and prostate tumor from Caucasians. Values normalized to BPH.

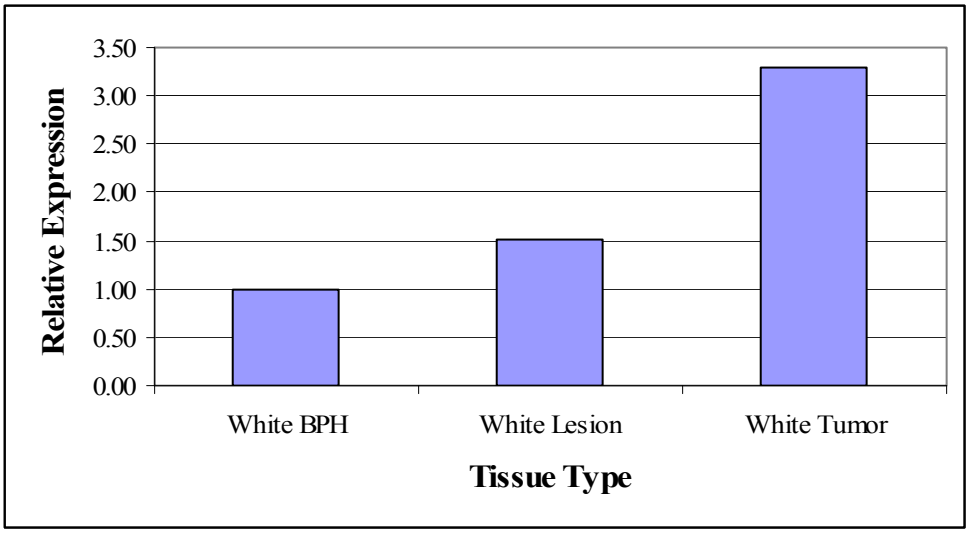

Figure 6. Relative expression levels of CYP1B1 in prostate tumor from African-American and Caucasians. Values normalized to African-Americans.

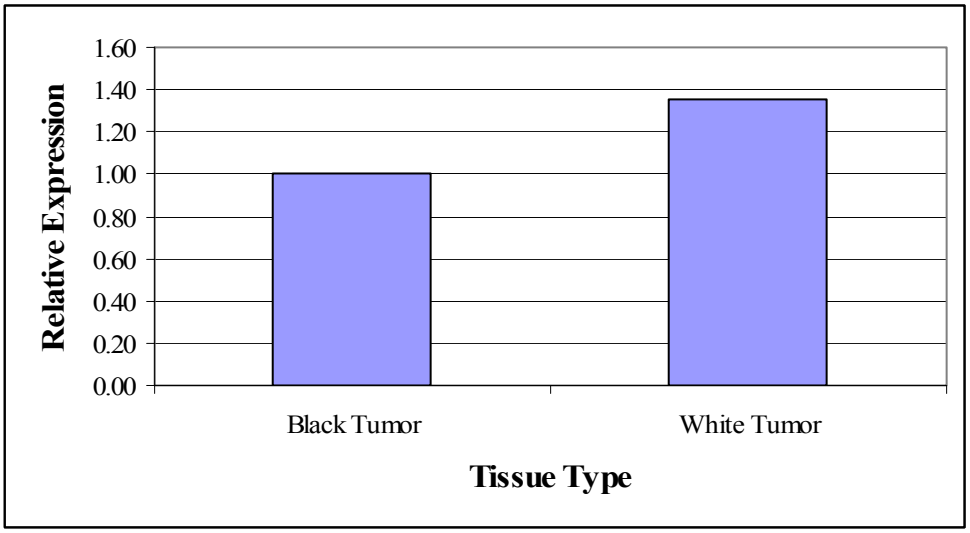

Figure 7. Immunohistochemistry staining of CYP1B1 protein in specimens from African-American prostate tumor (A), BPH (B), and normal adjacent region of tumor (C); and Caucasian prostate tumor (D), BPH (E), and normal adjacent region of tumor (F).

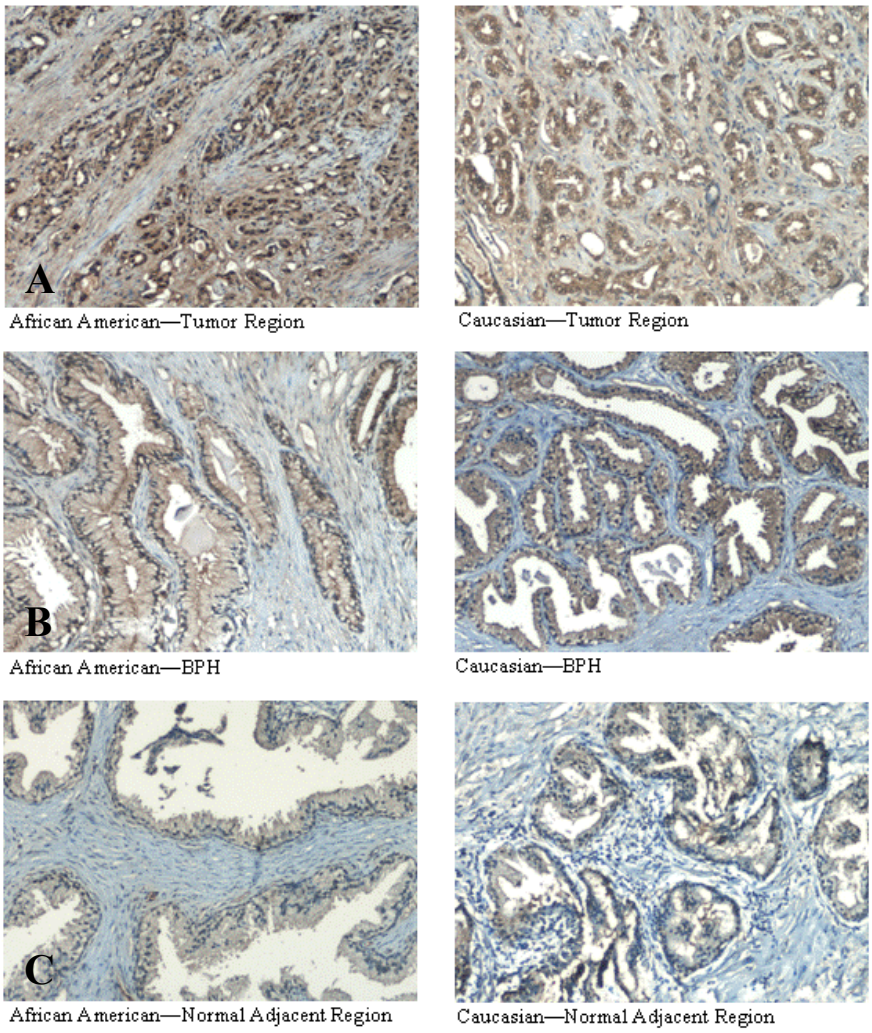

Table 1. Immunohistochemistry staining score of CYP1B1 protein in specimens of prostate tumor, BPH, and normal adjacent region of tumor (NAT) from African-Americans and Caucasians.

Tissue	CYP1B1 Score	Std.Dev.
Afr Am Tumor	1.57	0.87
Afr Am BPH	1.38	0.42
Afr Am NAT	1.32	1.00
White Tumor	1.50	0.60
White BPH	1.52	0.71
White NAT	1.20	0.73

Table 2. First step PCR and SSP prim ers utilized to determ ine SNP in healthy control, BPH and prostate cancer samples

CODON 119

1st PCR

Primer	Sequence		Anneal Temp
C119-Rev	ccttccagtgctccgagtag		
C119-For	ccccatagtggtgctgaatg	47	C

SSP

Primer	Sequence	Anneal Temp
C119 G-Rev with C119-For	acggaaggaggcgaaggc	65 C
C119 T-Rev with C119-For	acggaaggaggcgaagga	65 C

CODON 432

1ST PCR

Primer	Sequence	Anneal Temp
C432-Rev	tcatcactctgctggtcagg	
C432-For	gtcttgggctaccacattcc	47 C

SSP

Primer	Sequence	Anneal Temp
C432 C-Rev with C432-For	tccgggttaggccacttcag	65 C
C432 G-Rev with C432-For	tccgggttaggccacttcac	65 C

CODON 449

Determined by direct sequencing using 1ST PCR primers of codon 432 (C432-Rev and C432-For)

CODON 453

1ST PCR

Primer	Sequence		Anneal Temp
C453-Rev	agaaagttcttcgccaatgc		
C453-For	gacccactgaagtggcctaa	47	C

SSP

Primer	Sequence	Anneal Temp
C453 A-Rev with C453-For	tctgctggtcaggtccttgt	64 C
C453 G-Rev with C453-For	tctgctggtcaggtccttgc	64 C

Table 3. Genotypic frequencies of CYP1B1 SNPs between races for BPH and Prostate cancer and Total Patients. P-value reflect chi-square test.

Type	Codon	Gene	White	Black	OR	(95% CI)
BPH	119	G/G	21	16	Ref	
G/T			12	17	1.86	(0.71-4.88)
T/T			6	5	1.09	(0.29-4.19)
	432	C/C	11	2	Ref	
P<0.001		C/G	20	14	3.85	(1.01-14.62)
G/G			8	22	15.13	(4.14-55.23)
	453	A/A	28	33	Ref	
P<0.1		A/G	11	4	0.31	(0.10-0.95)
G/G			0	1	-	
PC	119	G/G	38	33	Ref	
P<0.001		G/T	14	34	2.80	(1.34-5.83)
T/T			7	30	4.94	(2.21-11.00)
	432	C/C	23	7	Ref	
P<0.001		C/G	22	30	4.48	(1.83-10.99)
G/G			14	60	14.08	(5.83-34.01)
	453	A/A	37	95	Ref	
P<0.001		A/G	19	2	0.04	(0.02-0.11)
G/G			3	0	-	
Total Patients (Includes Healthy Blacks)	119	G/G	59	78	Ref	
	P<0.001	G/T	26	93	2.71	(1.61-4.55)
		T/T	13	61	3.55	(1.96-6.43)
	432	C/C	34	17	Ref	
P<0.001		C/G	42	82	3.90	(2.03-7.52)
G/G			22	133	12.09	(5.95-24.58)
	453	A/A	65	214	Ref	
P<0.001		A/G	30	17	0.17	(0.09-0.34)
G/G			3	1	0.10	(0.01-1.02)

Table 4. Allele frequencies of CYP1B1 SNPs between races for BPH and Prostate cancer. P-value reflect chi-square test.

Type	Codon	Allele	White	Black
BPH	119	G	54	49
		T	24	27
	432	C	42	18
P<0.001		G	36	58
	453	A	67	70
		G	11	6
PC	119	G	90	100
P<0.001		T	28	94
	432	C	68	44
P<0.001		G	50	150
	453	A	93	192
P<0.001		G	25	2
Total Patients	119	G	144	249
(Includes Healthy	P<0.001	T	52	215
Blacks)				
	432	C	110	116
P<0.001		G	86	348
	453	A	160	445
P<0.001		G	36	19

Table 5. Genotypic and allelic frequencies of CYP1B1 SNPs between normal healthy and prostate cancer patients among Blacks. P-value reflect chi-square test.

Type	Codon	Gene	Healthy	Cancer	OR	(95% CI)
Genotype	119	G/G	29	33		
		G/T	42	34	0.71	(0.36-1.39)
		T/T	26	30	1.01	(0.49-2.09)
	432	C/C	8	7		
		C/G	38	30	0.90	(0.29-2.76)
		G/G	51	60	1.34	(0.46-3.94)
	449	C/C	9	10		
		C/T	36	33	0.83	(0.30-2.27)
		T/T	52	54	0.93	(0.35-2.47)
	453	A/A	86	95		
P=0.035		A/G	11	2	0.16	(0.05-0.51)
		G/G	0	0		-
Allele	119	G	100	100		
		T	94	94		
	432	C	54	44		
		G	140	150		
	449	C	54	53		
		T	140	141		
	453	A	183	192		
P=0.024		G	11	2		

Table 6. Linkage disequilibrium among 4 polymorphic sites of CYP1B1 in healthy controls of Black volunteers. D-values are shown.

Codon	119	432	449	453
119	-	0.0195	0.0305	-0.0181
432	0.0195	-	**0.1905**	0.0409
449	0.0305	**0.1905**	-	0.0409
453	-0.0181	0.0409	0.0409	-

Table 7. Haplotype frequencies of codons 432 and 449 of CYP1B1 among Black healthy controls and prostate cancer patients.

Haplotype	Control	Cancer	P-value
432G-449T	71.1%	71.0%	
432C-449C	26.8%	21.0%	
432G-449C	1.0%	6.3%	0.016
432G-449T	1.0%	1.6%	

Table 8. Genotypic frequencies of CYP1B1 SNPs between stages of cancer (< T2c vs ≥ T2c) for Blacks and Whites. Note: partial # of Black samples. P-value reflect chi-square test.

Race	Codon	Gene	< T2c	≥ T2c	P-value
Blacks	119	G/G	2	10	
		G/T	3	6	
		T/T	5	4	0.17
	432	C/C	2	1	
		C/G	2	5	
		G/G	6	14	0.43
	453	A/A	10	18	
		A/G	0	2	
		G/G	0	0	0.59
Whites	119	G/G	15	19	
		G/T	6	7	
		T/T	3	3	0.96
	432	C/C	12	9	
		C/G	8	13	
		G/G	4	7	0.37
	453	A/A	15	17	
		A/G	7	11	
		G/G	2	1	0.64

Table 9. Genotypic frequencies of CYP1B1 SN Ps between grades of cancer (< 7 vs > _ 7) for Blacks and Whites. Note: partial # of Black samples. P-value reflect chi-square test.

Race	Codon	Gene	< 7	≥ 7	P-value
Blacks	119	G/G	7	10	
		G/T	5	5	
		T/T	5	5	0.86
	432	C/C	1	2	
		C/G	5	4	
		G/G	11	14	0.76
	453	A/A	16	19	
		A/G	1	1	
		G/G	0	0	0.99
Whites	119	G/G	20	17	
		G/T	11	3	
		T/T	4	3	0.27
	432	C/C	14	9	
		C/G	15	7	
		G/G	6	7	0.44
	453	A/A	22	14	
		A/G	11	8	
		G/G	2	1	0.95

KEY RESEARCH ACCOPLISHMENTS:

- Evaluated CYP1B1 RNA expression in prostate cell lines.
- Evaluated CYP1B1 RNA expression in BPH and prostate cancer samples.
- Evaluated CYP1B1 protein in BPH and prostate cancer samples.
- Evaluated CYP1B1 SNPs in healthy controls, BPH and prostate cancer samples.
- Evaluated linkages between SNP sites.
- Evaluated SNP haplotypes between cases and controls.
- Evaluated SNPs in stages and grade of prostate cancer samples.

REPORTABLE OUTCOMES:

Abstract presented at the American Association for Cancer Research (April, 2006).
The significances of the research performed to date are the following:

1) CYP1B1 RNA expression is higher in cancer ous com pared to norm al prostate cell lines from Caucasian.
2) CYP1B1 RNA expression is higher in pros tate cancer tissue com pared to BPH from Caucasian.
3) CYP1B1 protein is localized in the cytoplas m of prostate cancer cells with som e expression in smooth muscle cells.
4) CYP1B1 protein expression on average is hi gher in prostate tum or region com pared to normal adjacent region.
5) CYP1B1 codon 119 T variant genotype and allele are significantly higher in African-Americans with prostate cancer compared to Caucasians.
6) CYP1B1 codon 432 G variant genotype and a llele frequency are significantly higher in African-Americans compared to Caucasians with BPH or prostate cancer.
7) CYP1B1 codon 453 G variant genotype and a llele frequency are significantly lower in African-Americans compared to Caucasians with prostate cancer.
8) In African-Americans, no associations found for prostate cancer at codons 119, 432, and 449 SNP sites. However, the G variant at codon 453 played a protective role against cancer.
9) In African-Am ericans, polym orphisms at codons 432 and 449 were in linkage disequilibrium.
10) In African-Americans, the 432G-449C haplotype is observed to be associated with prostate cancer.
11) CYP1B1 polymorphisms did not correlate with stage or grade of prostate cancer in African-Americans and Caucasians.

CONCLUSIONS:

CYP1B1 expression is higher in prostate cancer compared to BPH tissue or normal cells.
Racial differences in polym orphisms of the CYP1 B1 gene exist and therefore, m ay identify the population with higher risk for prostate cancer.

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APPENDICES:

Abstract presented at the American Association for Cancer Research in April, 2006.

#3687 Cytochrome P450 1B1 polymorphisms in African-Americans a Caucasians with prostate cancer. Yuichiro Tanaka, Hiroshi Hirata, Toshifu Kawakami, Deepa Pookot, Zhong Chen, Shinji Urakami, Ken Kawamoto, Hi Enokida, Rajvir Dahiya. *VA Medical Center, San Francisco, CA.*

Differential rates in incidence and mortality due to prostate cancer occur betwe races. A factor that may play a role in the carcinogenesis process is catechol-estrog and 4-hydroxy-estrogen has been shown to be tumorigenic and mutagenic. A enzyme capable of producing 4-hydroxy-estrogen is cytochrome P450 (CYP) 1 and polymorphisms of this enzyme have been demonstrated to be a risk factor f prostate cancer. We thus hypothesize that CYP1B1 polymorphisms could contri ute to racial differences in prostate cancer risk. To test this hypothesis, the genet distribution of three different CYP1B1 polymorphisms at codons 119(G→T 432(C→G), and 453(A→G), and their association with prostate cancer in Af can-American and Caucasian populations were investigated by using a sequenc specific PCR technique. For each race, prostate cancer and benign prostatic hyp plasia (BPH) control specimens were obtained. Results of preliminary experimen demonstrate the codon 119T, 432G and 453A alleles to be significantly higher i African-Americans compared to Caucasians among prostate cancer cases (P 0.01). When assessing the risk for prostate cancer as compared to BPH contro within races, no differences were observed for any of the polymorphic sites. Als differences were not detected between stages and grades of cancer. Thus, the experimental results demonstrate differences in CYP1B1 polymorphisms betwe races and may be important in understanding race-related prostate cancer.